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(54) Title: ULTRA HIGH PRESSURE, LOW TEMPERATURE FOOD PRESERVATION PROCESS (57) Abstract Provided here are methods for the commercial sterilization of foods that involve pre-heating the food to a temperature of about 110 °F to 160 °F, and thereafter pressurizing the food to 20,000 to 120,000 psi for a short period of time. One aspect of these methods is that sterilization can be achieved in a few minutes or less without exceeding the taste transition temperature of the food, thus retaining a fresh flavor. These methods are especially advantageous in preserving foods having a pH≤4.5. Also provided are methods of commercially sterilizing foods having a pH>4.5 by using ultra-high pressure to quickly reach the sterilization temperature of pre-heated canned foods.		

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ULTRA HIGH PRESSURE, LOW TEMPERATURE FOOD PRESERVATION PROCESS

Field of the Invention

5 This invention pertains to methods for achieving the commercial sterilization of foods having a $\text{pH} \leq 4.5$ involving concomitant treatment of the food with heat and high pressure.

Background of the Invention

10 The conventional approach to the commercial sterilization of food is thermal processing. Heating is highly effective in inactivating the microorganisms that cause food spoilage, but heating to sterilizing temperatures it also can cause some loss of nutrients and, for many foods, an undesirable change in flavor. This distinct change in flavor, often accompanied by a change in texture as well, occurs when the food reaches a certain critical temperature. The flavor change upon heating is particularly significant for fresh fruits and vegetables, and processed fruit and vegetable products.

15 Another aspect of food spoilage, especially in fruits, is discoloration, which is caused by endogenous enzymes such as polyphenyl oxidase or catalase. Another source of spoilage is the endogenous enzyme pectinase, which can cause a thinning or loss of viscosity that may be undesirable or unappealing. Thus, another goal of processing foods is the inactivation of these and other endogenous enzymes that may contribute to spoilage. Typically, the inactivation of undesirable endogenous enzymes is achieved by heating, but this heating often detracts from the fresh flavor of the food. Additives such as 4-hexylresorcinol, ascorbic acid, and phosphates are
20 also used to inactivate food-spoiling enzymes.

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A recently developed alternative to thermal processing is the use of ultra-high pressure to destroy spoilage-causing microorganisms and spoilage-related endogenous enzymes. The use of ultra-high pressure for food processing has been made possible by recent advances in the engineering of devices capable of delivering the necessary pressures to commercially useful amounts of foods. Machinery capable of industrial high pressure food sterilization is available, for example, from Flow International Corp. (Kent, WA), Mitsubishi Heavy Industries (Tokyo, Japan), Kobe Steel (Kobe, Japan), ABB Autoclave Systems, Inc. (Vasteras, Sweden), and Engineered Pressure Systems, Inc. (Andover, MA).

High pressures have been reported to have a variety of deleterious effect on bacteria and yeast. It has been shown, for example, to cause an increase in cell membrane permeability resulting in the loss of intracellular contents, and has been shown also to inhibit energy producing biochemical pathways and to denature various enzymes. In addition, high pressure is known to exacerbate the adverse effects of oxygen on the growth of anaerobic organisms.

One of the more demanding requirements of the commercial food preservation industry is the inactivation of bacterial spores, especially those of *Clostridium botulinum*. Bacterial spores are fairly resistant to heat inactivation unless extremely high temperatures are used. However, such temperatures adversely effect the quality of some foods. Pressures of approximately 44,000 pounds per square inch (e.g., 3,000 atmospheres) or greater have been shown to retard the germination of spores, possibly by denaturing enzymes involved in inducing spore germination. However, *C. botulinum* cannot propagate in foods having a pH \leq 4.5, thus such foods can be preserved without being subjected to the extreme treatments needed to inactivate *C. botulinum* spores.

Various applications of high-pressure food sterilization have been reported. For example, U.S. Patent No. 5,232,726 describes the use of ultra-high pressure homogenization as a means of prolonging the shelf life of orange juice. In this process, the orange juice is passed through a homogenization device under pressures of about 15,000 psi, resulting in juice that retained its fresh taste and remained palatable after being stored for up to forty days. In a brochure describing a ultra high-pressure isolator for in-line food processing, it was noted that foods can be preserved by exposure to pressures ranging from 50,000 psi to 100,000 psi for time periods ranging from 30 seconds to 2-3 minutes. (Flow International, Inc., Redmond, WA.). U.S. Patent No. 5,593,714 describes the sterilization and preservation of

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various fruits, vegetables, meats, and other food products by applying a pressure of about 25,000 psi for a period of five days. Moreover, pressure sterilization has been noted to be more effective in foods having an acidic pH than in other foods (see, e.g., Flow International brochure on ultra high-pressure isolator; U.S. Patent No. 5,316,745 at column 1; Morris, C.E., Food Engineering, October 1993, pp. 113-117).

Summary of the Invention

The present invention provides methods that combine ultra-high pressures and above-ambient temperatures to achieve the commercial sterilization of foods. In some embodiments of the invention, this is accomplished without exceeding the taste transition temperatures at which the foods may lose their fresh flavor. This method is particularly advantageous for foods having a $\text{pH} \leq 4.5$, as *C. botulinum* cannot propagate at such a pH. The methods described herein are equally applicable to foods with a pH greater than 4.5, provided these foods are treated also to eliminate *C. botulinum* spores.

In one aspect of the invention, foods to be sterilized are pre-heated, preferably to temperatures of about 100-160°F, and more preferably 110-160°F, before being subjected for about 10 seconds to 10 minutes to pressures of about 20,000-90,000 psi in a pressure chamber that itself has been pre-heated to the same initial temperature. The required duration of the pressurization step depends on the pH of the food and the temperature to which it is heated prior to pressurization. The application of pressure within this range of initial temperatures results in an instantaneous adiabatic temperature increase of about 15-40°F. The temperature increment that results from adiabatic heating is a function of both the initial temperature of the food and the amount of pressure applied. One aspect of adiabatic heating is that for a given pressure, the adiabatic heat increment increases as the initial pre-pressurization temperature increases. The highest temperature to which foods are subjected during the pressurization step can thus be controlled to ensure that the temperature during pressurization does not exceed the fresh flavor taste transition temperature. For most foods having a fresh flavor taste transition temperature, e.g., fruits such as strawberries, pineapple, and orange juice, the taste transition temperature is in the range of about 155-175°F, thus food treated according to the subject methods is held below these temperatures when preservation of fresh flavor is desired. In another aspect of the invention, the taste transition temperature for an individual food is

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determined empirically by tasting the food after heating it to different temperatures, and thereafter the food is not permitted to exceed this temperature during processing.

In other aspects of the invention, a food is flash-pressurized at very high pressures for a short time without preheating, e.g., at 100,000 psi for 10 seconds, to
5 inactivate enzymes that otherwise would cause browning or spoilage, and thereafter is processed at a lower pressure at a moderate temperature to inactivate spoilage-related microorganisms.

Detailed Description of the Preferred Embodiment

Provided here are methods for achieving the commercial sterilization of foods
10 that combine moderate temperatures with ultra-high pressures. These methods are especially advantageous for foods having a pH of 4.5 or less, since *C. botulinum* cannot propagate in such foods, hence these foods can be sterilized under less extreme conditions than non-acidic foods. A food rendered commercially sterile according to the subject methods has a shelf life of 3-24 months if stored at room
15 temperature, and may remain edible for much longer periods if stored refrigerated.

The subject methods involve heating a food to a temperature of between 100°F and 160°F, preferably to a temperature of 110°F to 160°F, and still more preferably to a temperature of between 120°F and 140°F, and subsequently
20 pressurizing the food. An important aspect of the disclosed methods is that they harness the adiabatic temperature increase that occurs when a food is subjected to ultra-high pressures. This adiabatic temperature increase is taken into account to ensure that the food remains below its taste transition temperature during processing, when processing foods for which flavor preservation is desired. The food may be pre-heated, e.g., in a water bath, before being placed in the pressurization vessel, or
25 alternatively, it can be pressurized in a pressure vessel equipped with its own heater. For use with a pressure vessel that lacks its own heating capacity, the pressure vessel may be pre-heated to the desired initial temperature by pumping hot water through the pressure cell, or by some other convenient means. If the device is equipped with its own heater, the cell can be raised to the desired temperature, the food packets
30 placed in the cell for a time sufficient for the packets to equilibrate with the cell temperature, and then pressure applied. Pressurization equipment having both heating and cooling capacity for the pressure vessel is available, for example, from ABB Autoclave Systems.

During the pressurization, the principle of adiabatic heating results in an
35 increase of about 15-40°F in the temperature of the food, the actual increment being a

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function of both the initial temperature and the amount of pressure applied. For example, if 50,000 psi is used to pressurize food pre-heated to 120-140°F, the adiabatic temperature increase is about 20°F, but if 90,000 psi is used, the increase in temperature is higher, about 40°F. Thus, applying ultra-high pressure to a pre-heated
5 food results in an actual sterilization temperature that is about 15-40° higher than the temperature to which the food was initially pre-heated. By taking the adiabatic temperature increase into account, the temperature of the food can be kept below the taste transition temperature when flavor preservation is desired. Pre-heat temperature and pressure can be selected in accordance with the present invention to reach a
10 maximum temperature under pressure of 140°F to 175°F, and preferably 150°F to 160°F, as determined for a particular food to avoid exceeding its fresh flavor transition temperature while still achieving commercial sterility.

Prior to the application of high pressure, air is removed from the food sample container and the pressurization vessel. If air were present during processing,
15 compounds that contribute to food flavor might become oxidized, and moreover, because air compresses at high pressures, its presence in the vessel would result in a loss of efficiency.

One aspect of high pressure food processing is the high cost of purchasing and maintaining equipment capable of delivering the requisite pressures. Stress on
20 the equipment, particularly the seals of the pressure vessel, is a function in part of the amount of time for which the pressure is held. In one aspect of the invention, the power of ultra-high pressure to inactivate endogenous enzymes is harnessed, while at the same time minimizing the stress produced by holding such pressures for long periods of time. In this approach, the food is brought rapidly to a pressure of about
25 80,000-120,000 psi, held at this pressure for about 10 seconds, then the pressure is released. This "flash-pressurization" inactivates endogenous enzymes that cause spoilage. Following this flash-pressurization, the food is pressurized to about 20,000-50,000 psi for 2-3 minutes at a moderate temperature to inactivate microorganisms.

30 In other aspects of the invention, FDA-approved food preservatives are added to the product prior to treatment with heat and pressure. Such preservatives, for example, include about 0.1% of 4-hexylresorcinol, sodium benzoate, potassium sorbate, Alta-D (Hansen Laboratories), Nisin (Hansen Laboratories), or other preservatives. In the presence of preservatives, commercial sterility can be achieved

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under even less extreme conditions of pressure and temperature than when they are not present.

During the pressurization step, the pressure applied is optimally between 20,000 psi and 90,000 psi, and preferably is between 40,000 to 80,000 psi, and still
5 more preferably is between 40,000 to 65,000 psi. The pressure is maintained for a period of time sufficient to achieve commercial sterility at the temperature being used.

As defined herein, the term "commercial sterility" is used according to its usual meaning, and refers to a food product in which the numbers of spoilage-causing
10 microorganisms are reduced to a commercially acceptable level, i.e., levels sufficiently low such that the product will not spoil if the food is stored for a reasonable length of time at a normal ambient temperature. Thus, a treatment that results in a "commercially sterile" product is one that inactivates microorganisms of public health concern that are capable of reproducing in the food under normal
15 nonrefrigerated conditions of storage and distribution. It is understood that food treated in accord with the subject methods is handled and processed under sanitary conditions, and ordinarily will not contain excessive numbers of contaminating microorganisms. An important aspect of commercial sterility is that live microorganisms in low numbers may be present in a package of commercially sterile
20 food, but when the food is stored for reasonable lengths of time, the microorganisms will not grow, and the food will remain safe and palatable. Thus, a commercially sterile food is defined as one in which any pathogenic or food-spoiling microorganisms that may be present are present at such low levels that fermentation or microbial growth will not occur upon prolonged storage for reasonable lengths of
25 time at normal ambient temperatures, e.g., when stored for 13 to 24 months.

Specific microorganisms associated with the spoilage of acidic foods include various yeasts (e.g., *Candida parapsilosis*, *Zygosaccharomyces bailii*, *Candida krusei*, *Saccharomyces cerevisiae*), molds (e.g., *Aspergillus* spp.), and lactic acid bacteria (e.g., *Lactobacillus fructivorans*).

For purposes of this invention, commercial sterility means specifically that 1
30 ml of a liquid food, e.g., juice or tea, or an aqueous suspension of a homogenate of a solid food (1:10, weight:volume) contains no more than 50 colony-producing yeast cells, no more than 100 colony-producing lactic acid bacteria, and less than 10 colony-producing *E. coli*, i.e., undetectable levels of *E. coli*. This latter
35 microorganism must be inactivated not because it causes spoilage, but because it is

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considered a health hazard. Experience has shown that if a product meets these criteria, no fermentation, i.e., no spoilage, will occur when the food is stored for a reasonable length of time, e.g., for 3 months and up to 1-2 years, when stored at ambient temperatures of 60°F-80°F. Thus, these levels of these microorganisms are considered commercially acceptable.

Pressurization for purposes of the subject methods may be achieved using any commercially available device capable of delivering the requisite high pressures. Prior to pressurization, the food product usually is sealed inside a suitable container such as a plastic bag, can, or other container, or may be pumped into the pressure vessel in bulk and packaged in sterile containers after the pressurization step.

The subject methods are particularly useful for preserving foods when a "cooked" flavor is not desired. Examples of such foods include fruit juice, jams and jellies, pie fillings, salads such as potato salad and bean salad, as well as pickles and sauces, such as ketchup. Other foods suitable for sterilization using the subject methods include canned and bottled tea without preservatives, and soda pop. The subject processes are especially advantageous for retaining a fresh flavor in foods that undergo a taste transition, i.e., a distinct loss of fresh flavor, when heated above a certain temperature called the "fresh flavor transition temperature." Acidic foods subject to this loss of flavor include most fruits, for example, cherries, strawberries, blueberries, pineapple, tomatoes, as well as cucumber pickles, orange juice, as well as many others. Sterilization temperatures provided by the subject methods, i.e., temperatures foods reach while being pressurized, are about 140-175°F, which temperatures are relatively low in comparison to temperatures used for conventional heat sterilization (180-210°F).

An important aspect of the subject method is that the highest temperature to which the food is heated, i.e., the temperature reached during pressurization, is selected to be below that of the fresh flavor taste transition temperature for particular foods, thus avoiding a "cooked" flavor in the processed food. For many foods, the taste transition temperature is between 155-175°F, and more commonly is between 160-170°F. If the taste transition temperature for a food is unknown, it can be readily determined by tasting the food after heating it to increasingly higher temperatures. Most fruits undergo a taste transition if heated above 160-170°F, as many of the compounds that contribute to flavor are shared by a variety of fruits. Once the transition temperature for a particular food is thus known, optimal sterilization conditions for that food can be determined by selecting a combination of pre-heating

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temperature and pressure such that the ultimate temperature reached when pressure is applied will not exceed the taste transition temperature for that food. Many commercially available pressure sterilization devices are equipped with a temperature probe to measure pressure cell temperature, thus by using one of these devices, the pressure chamber temperature can be accurately monitored. If a device not equipped with a temperature probe is being used, it can be assumed that a temperature increase of about 30°F will occur when pressure in the range of 40,000-60,000 psi is applied. The length of time required to achieve sterilization under these empirically-determined conditions is determined by holding the conditions for various lengths of time, then ascertaining the number of viable microorganisms remaining in the food by standard microbiological analysis, as illustrated in the Examples given below.

To readily ascertain the optimal length of the pressurization period for a selected combination of temperature and pressure, samples of the food can be spiked with large numbers of spoilage-related microorganisms prior to pressurizing the test samples. The shortest pressurization time that yields a commercially sterile product is optimal, as minimizing the processing time best preserves flavor and also advantageously speeds up production, while minimizing wear on the equipment. Thus, unlike conventional thermal sterilization, temperatures used in the subject methods are optimized to ensure that the flavor transition temperature is not exceeded, and the overall heating period is kept to a minimum.

In another aspect of the invention, the pressure vessel is pre-chilled, thus permitting the initial temperature of the food to be raised to a higher level, while still remaining below the taste-transition temperature of the food during pressurization.

The subject methods provide a means of preparing canned tea having the same high quality as tea packaged in glass bottles, which according to current practices is generally heat pasteurized. Moreover, the subject methods are especially advantageous for the sterilization of soda pop, as soda pop bottles are not amenable to being filled at high temperatures.

In another aspect of the invention, the subject methods are used to prepare uncooked fresh-tasting pie fillings that do not require any baking. Pie fillings having a pH≤4.5, particularly fruit fillings, are subjected to combined heat and pressure to reduce microorganism viability to an acceptable level, but without exceeding the taste transition temperature of the filling. Fillings thus prepared can be packaged for long-term storage at room temperature, and later can be placed into a pre-made pie crust and eaten without any requirement for baking. Such fillings have a fresh

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uncooked flavor, in contrast with the cooked pie fillings presently available in cans. Examples of fruits from which such fillings can be made are apples, strawberries, peaches, apricots, pears, blueberries, raspberries, blackberries, pineapples, tropical fruits, and other fruits.

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EXAMPLE 1

Pressurization at 60,000 psi at Various pHs, Times and Temperatures

This series of trials tested the effectiveness of combining pressurization at 60,000 psi with various values for pH, time, and temperature. Specifically tested were pH 3.5, 4.0, and 4.5, times of two and three minutes, and temperatures of 130°F and 140°F.

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The exemplary food used for these tests was strawberry jam, which contained 56.9% fresh frozen strawberries, 36.6% sugar, 5.8% water, 0.5% low methoxy pectin, and 0.2% lemon juice. A pH of about 3.4 is typical for strawberry jam. For these trials, the jam was adjusted with sodium bicarbonate to the pH to be tested, and 1-2 ounces of the jam were sealed in a number of individual plastic bags from which the air was evacuated prior to sealing. Using two bags per test variable, the bags were subjected to the conditions described below. Before being pressurized, the sealed bags were pre-heated to the test temperature by being placed in a hot water bath for about five minutes.

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Pressurization to 60,000 psi was accomplished using an ultra high pressure isolator (UHP) from Flow International Corporation, Kent, WA. Eight to ten sealed bags of jam were loaded per run, which filled the pressurization vessel. Prior to placing the bags in the pressurization chamber, the chamber walls were pre-heated to the target temperature by circulating hot water through the pressurization cell. As the walls of the pressure chamber are several inches thick, the walls, once heated, act as an effective heat sink, thus maintained a constant chamber temperature for the times required to process the bags. After loading the pre-heated pressure cell with the pre-heated sealed bags, the cell was filled with water pre-heated to the test temperature in order to eliminate air from the vessel. The pressure was then raised to 60,000 psi for either 2 minutes or 3 minutes. For samples having an initial temperature of 130°F, this amount of pressure results in an instantaneous increase in temperature of about 15°F, due to the principle of adiabatic heating, and for bags with an initial temperature of 140°F, the pressurization results in an increase in temperature of about 20°F. The initial pre-heating temperatures were selected such that upon pressurization the temperature of the jam was not expected to exceed the flavor

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transition temperature of the jam, which occurs at about 160°F. As controls, some of the bags of jam were neither heated nor pressurized, and some were pressurized without first being pre-heated.

5 After being allowed to cool, some of the plastic bags were opened and the processed jam was tested for flavor. No change in flavor was observed for any of the samples when compared with the taste of the untreated controls, thus indicating that temperatures reached during the pressurization step indeed did not exceed the taste transition temperature for strawberry jam, thus verifying the reliance on adiabatic heating principles for determining acceptable levels of pressure for pre-heated foods.

10 Two to three days after the processing, samples were analyzed as follows for the presence of viable microorganisms. Eleven grams of jam from each bag were separately weighed and used to prepare 1:10 dilutions by adding 99 ml of sterile 0.1% base peptone dilution water (0.1% Bactopeptone in distilled water; Difco, MI). The 1:10 dilution was used in turn to prepare ten-fold serial dilutions of 1:100 and
15 1:1000 in sterile base peptone dilution water. To assay for yeast and mold, 1 ml of each dilution was added to a 3M Yeast and Mold Petrifilm. The Petrifilms were incubated for 3 days at room temperature (20-23°C), followed by 3 days at 30°C. To assay for lactic acid bacteria, 1 ml of each dilution was added to a sterile petri dish, then 15-20 ml of melted sterile MRS agar (Difco, MI) that had been cooled to 50°C
20 was added to each dish and mixed thoroughly with the diluted sample. The petri dishes were incubated under anaerobic conditions for 3 days at 30°C in BBL jars with GasPak Plus™ envelopes (Baltimore Biological Laboratories, MD). For both types of assay, colonies were counted at the end of the stated incubation period. Microbiological analyses were performed on controls that had been either heated or
25 pressurized, but not on controls that were neither heated nor pressurized.

The results of these microbiological analyses are shown in Table 1, in which microbial growth is indicated by "yes" or "no." A "yes" in Table 1 indicates that less than 50 colony-forming yeasts and less than 100 colony-forming lactic acid bacteria were detected in the sample.

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Table 1

	Temp	Time	Microbial Growth
Pressure = 60,000 psi pH = 4.5	RT	0	yes
	RT	2 min	no
	RT	3 min	no
	130°F	2 min	no
	130°F	3 min	no
	140°F	2 min	no
Pressure = 60,000 psi pH = 4.0	140°F	3 min	no
	RT	0	yes
	RT	2 min	no
	RT	3 min	no
	130°F	2 min	no
	130°F	3 min	no
Pressure = 60,000 psi pH = 3.3	140°F	2 min	no
	140°F	3 min	no
	RT	0	no
	RT	2 min	no
	RT	3 min	no
	130°F	2 min	no
	130°F	3 min	no
	140°F	2 min	no
	140°F	3 min	no

Table 1 illustrates that for samples having a pH of either 4.5 or 4.0, pressurization without heat for at least 2 minutes at 60,000 psi was sufficient to provide commercial sterility. None of the samples, regardless of pH, that were both heated and pressurized exhibited significant microbial growth. However, the results

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of the pH 3.3 trials were difficult to interpret because even the sample that was neither heated nor pressurized appeared to be free of significant microbial growth.

Although the data in Table 1 suggest that pressurization alone reduces spoilage-related microorganisms to acceptable levels, it was apparent from the absolute numbers of microorganisms detected that fewer microorganisms had survived in the heated samples than in the unheated ones. For example, in the unheated pH 4.0 sample pressurized for 2 minutes, 40 yeast colonies were counted, in the corresponding sample heated to either 130°F or 140°F, <10 yeast colonies were observed ("<10" means that no colonies were observed in the 1 ml that was plated of the 1:10 dilution). In general, however, the results for the pH 3.3 samples suggested that insufficient numbers of microorganisms were present in the initial samples of jam to permit a fair assessment of the efficacy of combining moderate heat and high pressure.

Concomitant with the tests described above, also tested in this round of trials were a set of jam samples identical to the ones discussed above, but which were inoculated with large numbers of spoilage-causing microorganisms prior to being sealed in the plastic bags. The inocula per bag consisted of approximately 1.7×10^4 lactobacilli (the lactic acid bacteria mixture included *Lactobacillus fructivorans*, isolated lactics from salad dressings, and environmentally isolated lactics), and 3.2×10^4 yeasts ("yeasts" were a mixture of *Candida parapsilosis*, *Zygosaccharomyces bailii*, *Candida krusei*, *Saccharomyces cerevisiae*, yeast isolated from avocado, and yeast isolated from salad dressing), and 3.6×10^2 mold. The inoculated samples were heated and pressurized and analyzed for microbial growth exactly as for the samples described above. The numbers of microorganisms that survived in the inoculated samples are shown in Table 2.

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Table 2

	Temp	Time	Yeast	Mold	Lactics
Pressure=60,000 psi	RT	0	2,300	40	26,000
	RT	2 min.	1,660	20	14,000
	RT	3 min.	990	30	12,600
pH 4.5	130°F	2 min.	40	10	40
	130°F	3 min.	<10	<10	<30
	140°F	2 min.	<10	<10	<10
	140°F	3 min.	10	<10	<10
	RT	0	1,700	20	25,000
	RT	2 min.	2,100	40	21,600
Pressure=60,000 psi	RT	3 min.	860	30	9,800
pH 4.0	130°F	2 min.	<10	<10	40
	130°F	3 min.	<10	<10	30
	140°F	2 min.	<10	<10	<10
	140°F	3 min.	<10	<10	<10
	RT	0	1,000	30	9,000
	RT	2 min.	310	40	440
Pressure=60,000 psi	RT	3 min.	160	20	<10
pH 3.3	130°F	2 min.	<10	<10	<10
	130°F	3 min.	<10	<10	<10
	140°F	2 min.	<10	<10	<10
	140°F	3 min.	<10	<10	<10

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5 The data in Table 2 clearly illustrate that at all three pHs tested, the combination of pressure at 60,000 psi with moderate temperatures for brief periods of time vastly reduced the numbers of viable microorganisms as compared with the numbers of live microorganisms in unheated samples that were subjected to pressure alone. The results of Table 2 indicate that food-spoiling microorganisms are reduced to acceptable levels at $\text{pH} \leq 4.5$ by pressuring at 60,000 psi for at least 2 minutes after pre-heating to at least 130°F.

10 The results shown in Table 2 showed little difference in results whether the pressurized samples were heated for either 2 minutes or 3 minutes. These data thus suggest that by using heat and high pressure in conjunction, satisfactory results can be obtained using pressurization periods as short as two minutes, and possibly even shorter. This is an important advantage of the subject invention, as commercial pressurization devices are very costly, and a commercially viable process must provide rapid processing to maximize the efficient use of this equipment.

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EXAMPLE 2

Pressurization Under Various Conditions of pH and Temperature

20 This set of trials was conducted to further explore the efficacy of combining moderate temperatures and very brief periods of high pressure to achieve commercial sterility. The variables tested here were pressure, pH, time, and temperature. Test conditions included: temperature, 120°F and 140°F; pressure, 40,000, 60,000, and 65,000 psi; pH, 3.4 and 4.5; pressurization periods, 0, 10, 30, 60, and 120 seconds. For each set of test conditions, three sealed packets containing 1-2 ounces of strawberry jam base were prepared exactly as described in Example 1, two of which were used as controls. One of the controls was neither heated nor pressurized, and
25 the other was heated but not pressurized.

Processing of the samples and microbiological analyses were conducted as described for Example 1, except that here the inoculated jam samples were inoculated with *E. coli* in addition to the yeast, mold, and lactics. The inocula used in these tests were 1.7×10^5 yeasts, 4.5×10^6 lactics, 2.2×10^2 mold, and
30 9.1×10^4 *E. coli* per bag of jam. To assay for *E. coli* in these samples, 1 ml of each dilution was added to 3M Coliform Petrifilm, and incubated at 35°C for 48 hours before colonies were counted. Baseline assays performed prior to inoculating with *E. coli* indicated that no coliforms were present in the initial jam samples. All of the heated or heated and pressurized samples in this series retained a fresh flavor after
35 treatment. All of the non-heated, non-pressurized control bags, both inoculated and

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uninoculated, became swollen and burst by 3 days of incubation at room temperature. Specific sets of conditions tested and the results of the microbiological analyses for the uninoculated samples are shown in Table 3.

Table 3

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	Pressure	Temp	Time	Microbial Growth
pH = 4.5	ambient	RT	0	yes
	ambient	120°F	0	no
	ambient	140°F	0	no
	60,000 psi	120°F	10 sec	no
	60,000 psi	120°F	30 sec	no
	60,000 psi	140°F	10 sec	no
	60,000 psi	140°F	30 sec	no
	65,000 psi	120°F	10 sec	no
	65,000 psi	120°F	30 sec	no
	65,000 psi	140°F	10 sec	no
	65,000 psi	140°F	30 sec	no
pH = 3.4	ambient	RT	0	yes
	40,000 psi	140°F	60 sec	no
	40,000 psi	140°F	120 sec	no
	65,000 psi	120°F	10 sec	no
	65,000 psi	120°F	30 sec	no
	65,000 psi	140°F	10 sec	no
	65,000 psi	140°F	30 sec	no
	65,000 psi	140°F	30 sec	no

As for the first set of trials (Example 1), all of the uninoculated samples in this set of trials appeared to be commercially sterile except for controls that were neither heated nor pressurized.

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A second group of jam samples were tested under conditions identical to those shown in Table 3, except that the samples prior to sealing were inoculated as described above with *E. coli*, yeasts, lactic acid bacteria, and mold, and the results obtained with these samples are shown in Table 4.

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Table 4

	Pressure	Temp	Time	Yeast	Mold	Lactics	<i>E. coli</i>
pH 4.5	ambient	RT	0	156,000	90	1,350,000	71,000
	ambient	120°F	0	23,000	20	390,000	2,200
	ambient	140°F	0	320	<10	340	<10
	60,000	120°F	0	23,000	20	390,000	2,200
	60,000	120°F	10 sec.	7,800	100	220,000	1,600
	60,000	120°F	30 sec.	60	<10	210,000	<10
	60,000	140°F	0	320	<10	120	<10
	60,000	140°F	10 sec.	20	<10	30	<10
	60,000	140°F	30 sec.	<10	10	40	<10
	65,000	120°F	10 sec.	6,300	100	220,000	1,400
	65,000	120°F	30 sec.	270	<10	210	90
	65,000	140°F	10 sec.	20	<10	520	<10
	65,000	140°F	30 sec.	<10	<10	10	<10
pH 3.4	ambient	RT	0	31,000	60	410,000	3,200
	ambient	120°F	0	15,000	10	126,000	1,570
	ambient	140°F	0	270	<10	120	<10
	40,000	140°F	60 sec.	<10	10	<10	<10
	40,000	140°F	120 sec.	<10	10	<10	<10
	65,000	120°F	10 sec.	1,370	10	210,000	380
	65,000	120°F	60 sec.	80	<10	530	<10
	65,000	140°F	10 sec.	<10	<10	430	<10
	65,000	140°F	60 sec.	<10	<10	190	<10

The data of Table 4 indicate the superiority of combining pressurization with heating to 120°F, as compared with only heating to 120°F. For example, at pH 4.5, heating to 120°F without pressurization reduced the yeast count from 156,000 to 23,000,

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whereas adding 30 seconds of 60,000 psi pressurization at that same temperature reduced the yeast count to 60, a number that meets the criterion for commercial sterility for this organism. *E. coli* were also reduced to undetectable levels under this set of conditions. However, an unacceptable number of lactics survived these conditions. When the pressurization step was preceded by heating to 140°F rather than 120°F, both yeast and lactics were reduced to acceptable levels at this pH. With pre-heating to 140°F, even 10 seconds of pressurization at 60,000 psi was adequate to reduce both yeasts and lactics to acceptable levels at pH 4.5. The results obtained with 65,000 psi are inconsistent with the results at 60,000 psi, and may have reflected an equipment failure or operator error during the 65,000 psi run.

For the pH 3.3 samples, better results were generally observed for those samples subjected to combined heat and pressure as compared with those that were heated without pressure (Table 4), although the level of lactics remained too high in many of the 65,000 psi samples. As mentioned above, it seems likely that some technical error occurred during the 65,000 psi run. However, the efficacy of the method is nonetheless confirmed by comparing the results obtained at 65,000 psi when the samples were pre-heated to 120°F with those obtained after pre-heating to 140°F. Clearly, fewer microorganisms survived in the latter samples. Of the pH 3.3 samples, those heated to 140°F at 40,000 psi for 60 seconds met the criteria for commercial sterility, thus suggesting that these conditions are satisfactory for achieving commercial sterility. In all of the pH 3.3 samples, the residual microorganisms in the heated and pressurized samples were substantially reduced as compared with the numbers present in the initial inocula, thus the pH 3.3 results taken as a whole indicate the superiority over heat alone of combining moderate temperatures with high pressure treatment for the commercial sterilization of low pH food products.

EXAMPLE 3

Combined Heating and Pressurization for Processing Foods with pH>4.5

The method of combining moderate temperatures with high pressure can be adapted to advantageously kill undesirable microorganisms in foods having a pH>4.5. Such foods, when sterilized by conventional thermal methods, typically are heated to 250°F for six to twelve minutes in order to kill all spores of *C. botulinum*, which is required to render such foods commercially sterile. Once heat is applied, it usually takes about an hour for canned products to reach this temperature, and another 30 minutes for the cans to cool to ambient temperature. Thus, the heating

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and cooling periods flanking the actual sterilization period contribute substantially to the "cooked" taste that results from conventional thermal sterilization.

In accordance with another embodiment of this invention, the adiabatic heat increase can be harnessed to achieve effective thermal sterilization while reducing the exposure of canned high pH foods to high temperatures. For this method, canned foods are heated to an initial temperature of 210°F, then pressurized to 60,000 psi, thus resulting in an instantaneous temperature increase to reach a sterilization temperature of about 240-250°F. The pressure is held for a time period sufficient to kill *C. botulinum* spores or other undesired microorganisms (e.g., from about 1 to 15 minutes), then returned to ambient pressure. The optimal time for the pressurization step is determined by using microbiological analysis to ascertain the survival of *C. botulinum* spores. Similarly, a final sterilization temperature of 250-270°F is achieved by heating the product to an initial temperature of 230-250°F, and subsequently subjecting the food to 60,000 psi. At temperatures of 250-270°F, the kill rate of microorganisms is very rapid, thus ten to 30 seconds is sufficient to achieve commercial sterility.

The method can be adapted for use with heat-sensitive foods. Heat-sensitive foods having a pH>4.5 are pre-heated to a temperature not exceeding about 210°F. Thus, when pressure to 60,000 psi is applied, the temperature is raised to about 235-240°F. These conditions are held for six to twelve minutes, then the pressure is released, resulting in the temperature instantaneously returning to 210°F. Because of the rapidity of the pressure-associated rise and drop in temperature, these methods reduce the duration of product exposure to high temperatures, thereby minimizing the deleterious effects of heat on flavor. This reduced exposure to heat is especially advantageous in the case of heat-sensitive foods, and also speeds net processing time.

By decreasing the amount of time canned food is subjected to above-ambient temperatures, the subject methods minimize the time period during which the food is "cooked," thus maximizing the preservation of flavor without compromising the sterility of the product.

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

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The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for achieving the commercial sterilization of a fresh food that has a pH of 4.5 or less, comprising:

heating the food to an initial temperature of at least 100°F;
pressurizing the pre-heated food to a pressure of at least 20,000 psi; and,
maintaining said pressure for a time sufficient to result in commercial sterility of the food.

2. The method of Claim 1, wherein the initial temperature of the food is from 110°F to 160°F.

3. The method of Claim 2, wherein the initial temperature of the food is from 120°F to 140°F.

4. The method of Claim 1, wherein the pressurization is maintained for a time period of between 10 seconds and 10 minutes.

5. The method of Claim 1, wherein the pressure is from 20,000 psi to 120,000 psi.

6. The method of Claim 5, wherein the pressure is from 40,000 to 80,000 psi.

7. The method of Claim 1, wherein air is removed from the food sample container and the pressurization vessel prior to the pressurization step.

8. The method of Claim 1, wherein a food preservative is added to the food prior to heating.

9. The method of Claim 8, wherein the food preservative is selected from the group consisting of 4-hexylresorcinol, sodium benzoate, potassium sorbate, Alta-D, and Nisin.

10. The method of Claim 1, wherein the food is selected from the group consisting of a fruit, a fruit juice, a jam, a jelly, a pie filling, a tea, a soda pop, a salad, a pickle, and a sauce.

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11. A method for achieving the commercial sterility of a fresh food that has a pH of 4.5 or less, the food having a fresh flavor transition temperature at which the flavor of the food transitions to a cooked flavor, comprising:

pre-heating the food to an initial temperature of at least 110°F, and less than the fresh flavor transition temperature of the food; and

pressurizing the pre-heated food to a pressure of at least 20,000 psi and less than a pressure at which adiabatic heating upon pressurization causes the temperature of the food to exceed the fresh flavor transition temperature of the food; and

maintaining the pressurization for a time sufficient to result in commercial sterility of the food.

12. The method of Claim 11, wherein the temperature of the food during pressurization is between 160°F and 170°F.

13. A method for achieving the commercial sterility of a fresh food that has a pH of 4.5 or less comprising:

pressurizing the food to a pressure of from 80,000 psi to 120,000 psi;

releasing the pressure; heating the food to a temperature of at least 110°F; and

pressurizing the heated food to a pressure of 20,000 psi to 50,000 psi for a time sufficient to result in commercial sterility of the food.

14. A method for achieving the commercial sterility of a fresh food that has a pH greater than 4.5 comprising:

heating the food to an initial temperature of at least 210°F;

pressurizing the food to a pressure of at least 60,000 psi; and

maintaining the pressurization for a time sufficient to result in commercial sterility of the food.

15. The method of Claim 14, wherein the time for which the pressurization is maintained is between 1 to 15 minutes.

INTERNATIONAL SEARCH REPORT

International Application No

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L3/015

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	ROVERE P: "Stabilisation of Apricot puree by means of High Pressure treatments" PREHRAMBENO - TEHNOLOSKA I BIOTEHNOLOSKA REVIJA, vol. 32, no. 4, 1994, pages 145-150, XP002096446 see the whole document	1-6, 10, 11
Y A	---	7 8,9
Y	PATENT ABSTRACTS OF JAPAN vol. 017, no. 266 (C-1062), 25 May 1993 & JP 05 007479 A (TOPPAN PRINTING CO LTD), 19 January 1993 see abstract ---	7

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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 97 21361 A (KAL KAN FOODS) 19 June 1997 see abstract see page 1, line 14 - page 8, line 23 see claims ---	14, 15 1-13
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